REMARKS/ARGUMENTS

Status of the Claims

Upon entry of the present amendment, claims 1, 3, 6, 7, 10-13, 34-36 and 38-41 are amended. Claims 2, 4-5, and 14-33 are canceled without prejudice to renewal. New claims 42-48 are added.

Claim 1 is amended to set forth a method for reducing expression of a mammalian SREBP-1 gene, and to set forth that the antagonist is an oxysterol. Support for administering an antagonist is found, for example, on page 9, lines 4-6 and 29-32. Support for oxysterol is found, for example, on page 9, lines 27-29, page 12, lines 23-25, page 19, lines 5-6, and page 27, lines 24-25.

Claim 3 is amended to set forth that the modulator inhibits LXRα-mediated expression.

Claim 6 is amended to correctly depend from pending Claim 1, and to correct antecedent basis by changing "modulator compound" to "antagonist."

Claim 7 is amended to depend from Claim 6.

Claims 10 and 11 are amended to eliminate recitation of the phrase "the modulator compound is an antagonist of LXRα and."

Claim 12 is amended to set forth that the modulator compound comprises at least one of LXRα antagonist and LXRα agonist activity that promotes or inhibits LXRα-mediated expression of an SREBP-1 gene. Support for agonist is found, for example, on page 5, line 32 through page 6, line 6; page 38, lines 6-9 and in Figure 8. Support for antagonist is found, for example, in originally filed claim 5, on page 9, lines 4-6 and 29-32, on page 10, lines 22-25 and on page 42, lines 24-25.

Claim 13 is amended to correct a typographical error.

Claims 34-36 and 38-41 are amended to set forth a method for ameliorating a condition associated with abnormally high SREBP-1 expression in a mammal and that the

antagonist is an oxysterol. Support is found, for example, on page 9, lines 27-29, page 12, lines 23-25, page 19, lines 5-6, and page 27, lines 24-25.

New claim 42 sets forth that the condition associated with abnormally high SREBP-1 expression is pancreatitis. Support is found, for example, on page 1, line 17.

New claim 43 sets forth that the modulator compound comprises an agonist of LXRα. Support is found, for example, in originally filed claim 2, on page 9, lines 9-11, and in Examples A and B on page 33, line 15 through page 34, line 8.

New claim 44 sets forth that the modulator compound promotes or inhibits LXRα-mediated expression of the SREBP-1c transcript. Support is found, for example, in originally filed claim 3, on page 4, lines 6-9, on page 9, lines 25-32, and in Example C on page 34, lines 9-16.

New claim 45 sets forth that the modulator compound is 24,25-epoxycholesterol. Support is found, for example, in Examples A and B on page 33, line 15 through page 34, line 8, and in Figures 2-3.

New claim 46 sets forth that the modulator compound comprises an antagonist of LXRα and inhibits LXRα-mediated expression of the SREBP-1 gene. Support is found, for example, in originally filed claim 5, on page 9, lines 4-6 and 29-32, and on page 42, lines 24-25.

New claim 47 sets forth a method of increasing triglyceride levels in a mammal by administering an effective amount of an agonist of LXRα. Support is found, for example, on page 5, line 32 through page 6, line 6; page 38, lines 6-9 and in Figure 8.

New claim 48 sets forth that the agonists are selected from the group consisting of an oxysterol, *N*-methyl-*N*-[4-(2,2,2-trifluoro-l-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide (T0314407), N-(2,2,2-trifluoro-ethyl)-*N*-[4-(2,2,2-trifluoro-l-hydroxy-1-trifluoromethyl-ethyl)-phenyl]-benzenesulfonamide (T0901317), and mixtures thereof. Support is found, for example, on page 37, lines 5-14 and in Figure 6A and 6B.

New claim 49 sets forth a method of decreasing triglyceride levels in a mammal by administering an effective amount of an antagonist of LXRα. Support is found, for example, in originally filed claim 5, on page 9, lines 4-6 and 29-32, and on page 42, lines 24-25.

Rejections under 35 U.S.C. § 112, first paragraph, enablement requirement

A. Recitation of "modulation"

The Examiner has rejected claims 1-13 and 34-41 as allegedly failing to meet the enablement requirements under Section 112, first paragraph, because the claims allegedly fail to recite a specific therapeutic goal or a specific therapeutic treatment. The Examiner further asserts that modulating expression, which encompasses promoting or inhibiting expression are actions in opposite directions. Claims 1, 12 and 34 are independent.

Applicants address this rejection also as it may apply to new claims 42-46.

Claims 1-11

In view of the amendment to independent claim 1, which now recites a method for reducing expression of a mammalian SREBP-1 gene, this rejection applied to claims 1-11 is rendered moot. Claim 1, and claims 3 and 6-11 which depend therefrom, set forth a specific therapeutic goal of reducing expression of a mammalian SREBP-1 gene by administering an antagonist of LXRα that inhibits LXRα-mediated expression of a SREBP-1 gene.

Claims 12-13 and 43-46

The Examiner objects to the recitation of "modulating" and "modulator," stating that promoting or inhibiting expression of a mammalian SREBP-1 gene are actions in opposite directions.

This rejection is respectfully traversed, first because amended independent claim 12 sets forth the specific therapeutic goal of modulating the triglyceride levels in a mammal. Whereas hypertriglyceridemia was a commonly understood pathological condition as of the May 3, 2000 priority date of the instant application, hypotriglyceridemia was also a recognized pathological condition as of May 3, 2000 (*see, for example*, Wang, *et al.*, (1995) *J Surg Res* 59:326; Brown (1995) *Med Hypotheses* 45:91; Maeda, *et al.*, (1994) *J Biol Chem* 269:23610; Gouache, *et al.*, (1991) *J Nutr* 121:653; and Camus, *et al.*, (1988) *Biochim Biophys*

Acta 961:53, attached as Exhibit A). Therefore, depending on the pathological condition of an individual, both raising abnormally low or lowering abnormally high triglyceride levels is a specific and legitimate therapeutic goal.

Second, the present invention has identified that molecules that function as agonists and/or antagonists of a LXRα receptor can be used to modulate triglyceride levels in a mammal, as needed. Actions of promoting and inhibiting are not entirely opposite in biological systems, because numerous drugs, including sterol receptor ligands, can act simultaneously as partial antagonists and partial agonists (*see, for example*, Somjen, *et al.*, (2004) *J Steroid Biochem Mol Biol* 91:147; Galbiati, *et al.*, (2002) *J Pharmacol Exp Ther* 300:802; Vaisanen, *et al.*, (2002) *J Mol Biol* 315:229; Bula, *et al.*, (2000) 14:1788; and Bryant, *et al.*, (1998) *Proc Soc Exp Biol Med* 217:45; attached as Exhibit B). Furthermore, chemically similar modulating compounds can bind to the same receptor and function as an agonist, an antagonist or both. For example, 27-hydroxycholesterol can function as an LXR antagonist (Davies, *et al.*, (2004) *J Biol Chem*, Nov. 16, attached as Exhibit C) and 24,25-epoxycholesterol can function as an LXR agonist.

Further, the instant specification provides extensive guidance to those of ordinary skill in the art to identify oxysterol antagonists and/or agonists of LXRα that modulate the expression of SREBP-1. For Example, Section A of the Preferred Embodiments, on page 9, line 16 through page 19, line 18, teaches direct and displacement assays to identify compounds that alter the interaction between LXRα and ligands of LXRα (Section A1, page 12, line 3 through page 17, line 14), and cell-based assays to identify compounds that modulate SREBP-1 expression (Section A2, page 17, line 15 through page 19, line 18). Using these assays, one can readily screen without undue experimentation any of a number of different compounds to identify compounds that modulate SREBP-1 expression.

Because amended independent claim 12 sets forth a specific therapeutic goal and because it is a common pharmacological phenomenon well known to those of ordinary skill in the art that a single compound can simultaneously function as an antagonist and an agonist, the

Examiner is respectfully requested to withdraw this rejection, as it applies to claims 12-13 and 43-46.

Claims 34-42

As this rejection applies to claims 34-41, Applicants respectfully traverse because independent claim 34 sets forth the specific therapeutic goal of ameliorating a condition associated with abnormally high SREBP-1 expression by administering a therapeutically effective amount of a LXRα antagonist. No undue experimentation is involved in practicing the methods of claims 34-42, because the pathological condition of abnormally high SREBP-1 expression can be clearly identified by those of skill in the art and the step for treating the identified pathological condition with an antagonist is also clear and predictable, with a reasonable expectation of success.

B. Recitation of "compound that promotes or inhibits LXRα-mediated expression of the SREBP-1 gene" or "LXRα antagonist"

The Examiner has rejected claims 1-3, 5-13 and 34-41 as not enabled for reciting the allegedly merely functional language of a "compound that promotes or inhibits LXRα-mediated expression of the SREBP-1 gene" in claims 1 and 12, or an "LXRα antagonist" in claim 34. Claim 4, which recites administering 24,25-epoxycholesterol, has not been included in this rejection.

Applicants render this rejection moot by amending claims 1 and 12 to set forth that the modulator compound is an oxysterol, and by amending claim 34 to recite that the LXRα antagonist is an oxysterol. The specification teaches the use of oxysterols in the claimed methods (*see, e.g.*, page 9, lines 27-29, page 12, lines 23-25, page 19, lines 5-6, and page 27, lines 24-25). The Examiner agrees that the present specification teaches the use of oxysterols in the present methods (*see*, page 7, lines 19-21 and page 9, lines 14-15 of the Official Action mailed September 24, 2004).

In view of the foregoing arguments, the Examiner is respectfully requested to withdraw these rejections.

Rejection under 35 U.S.C. § 102(a)

A. Alleged anticipation in view of Medina

The Examiner has rejected claims 1-3, 5-13 and 34-41 as allegedly anticipated by Medina, *et al.* (WO 99/10320) ("Medina"). Claim 4, which recites administering 24,25-epoxycholesterol, has not been included in this rejection.

This rejection is rendered moot by amending independent claims 1, 12 and 34 to set forth administering an oxysterol. For the same reason, this rejection does not apply to newly added independent claim 48.

This rejection does not apply to newly added claims 47 and 48, which are directed to methods of increasing triglyceride levels in a mammal and for promoting expression of a mammalian SREBP-1 gene. Medina does not disclose or suggest any substituted benzene compound that increases triglyceride levels in a mammal.

B. Alleged anticipation in view of Dollis

The Examiner has rejected claims 1-7 as allegedly anticipated by Dollis, *et al.* (1994) *Biochem Pharmacol* 48:49.

This rejection is rendered moot by amending independent claim 1 to set forth a method for reducing expression of a mammalian SREBP-1 gene by administering an antagonist of LXRα that inhibits LXRα-mediated expression. As taught in the instant specification, 24,25-epoxycholesterol promotes LXRα-mediated expression.

C. Alleged anticipation in view of Sato

The Examiner has rejected claims 1-7 as allegedly anticipated by Sato, *et al.* (1984) *Chem Pharm Bull* 32:3305.

This rejection is rendered moot by amending independent claim 1 to set forth a method for reducing expression of a mammalian SREBP-1 gene by administering an antagonist of LXRα that inhibits LXRα-mediated expression.

Double-Patenting Rejections

A. Claims 39-42 of U.S. Patent No. 6,316,503

The Examiner has rejected claims 1-3, 5-13 and 34-41 as allegedly obvious over co-owned U.S. Patent No. 6,316,503. Claim 4, which recites administering 24,25-epoxycholesterol, has not been included in this rejection.

This rejection is rendered moot by amending independent claims 1, 12 and 34 to set forth administering an oxysterol.

B. Claims 1, 17, and 25-27 of U.S. Patent No. 6,388,131

The Examiner has rejected claims 1-3, 5-13 and 34-41 as allegedly obvious over co-owned U.S. Patent No. 6,388,131. Claim 4, which recites administering 24,25-epoxycholesterol, has not been included in this rejection.

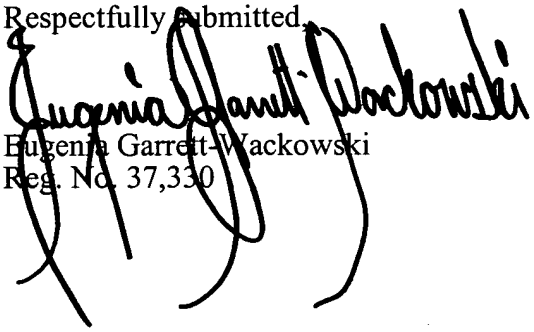
This rejection is rendered moot by amending independent claims 1, 12 and 34 to set forth administering an oxysterol.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



Eugenia Garrett-Wackowski
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1: J Surg Res. 1995 Sep;59(3):326-36.

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ELSEVIER SCIENCE
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Alterations of lipid contents in blood, hepatocytes, and enterocytes in the early stage of acute liver failure induced by 90% hepatectomy in the rat.

Wang X, Wang W, Bengmark S, Andersson R.

Department of Surgery, Lund University Hospital, Sweden.

Potential alterations in lipid metabolism in the early stage of acute liver failure are poorly elucidated. In the present study, acute liver failure was induced by subtotal hepatectomy (90%) in the rat in order to investigate early alterations in lipid contents in blood, hepatocytes, and enterocytes. Hypocholesterolemia and hypotriglyceridemia appeared 2 and 6 hr following subtotal hepatectomy. Plasma levels of high density lipoprotein-cholesterol were significantly lower in rats with acute liver failure than in controls, which may be associated with hypocholesterolemia. An increase in erythrocyte phospholipids and triglycerides was seen from 2 hr on after hepatectomy. The content of phospholipids and triglycerides was reduced in isolated enterocytes from the proximal small intestine and increased in enterocytes from the distal small intestine. Isolated hepatocytes from the remnant liver exhibited an increase in phosphatidylethanolamine and a decrease in phosphatidylinositol. Levels of enterocyte phosphatidylserine were elevated in both the proximal and the distal small intestine, while they diminished in the distal small intestine. Sphingomyelin content increased in the proximal small intestine. The recognition of lipid alterations in the intestine-liver-systemic circulation axis in the early stage of acute liver failure may be beneficial in improving the recovery from acute liver failure.

PMID: 7643590 [PubMed - indexed for MEDLINE]

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1: Med Hypotheses. 1995 Jul;45(1):91-3.

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Werner syndrome and genetic obesity: speculation.

Brown DW.

Werner syndrome (WS) is a rare autosomal recessive disease with poor growth, premature aging, scleroderma-like skin changes, endocrine abnormalities, and deficiencies of adipose tissue. Could there be a genetic obesity syndrome which offers an instructive contrast to at least one form of WS? At least one form of WS might result from an enzyme defect that causes hypertriglyceridemia, hyperinsulinism, and hyperglucagonism; the defective enzyme might play a key role in the utilization of tryptophan, riboflavin (vitamin B2), or other vitamins or in the synthesis of prostaglandins that inhibit insulin secretion. At least one form of genetic obesity might result from an enzyme defect that causes hypotriglyceridemia and hyperinsulinism without hyperglucagonism; the defective enzyme might be unable to bind properly to a product that inhibits some step in the process of conversion of free fatty acid (FFA) CoA into ketoacids.

PMID: 8524189 [PubMed - indexed for MEDLINE]

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1: J Biol Chem. 1994 Sep 23;269(38):23610-6.

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Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia.

Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J.

Department of Pathology, University of North Carolina, Chapel Hill 27599-7525.

Using gene targeting in embryonic stem cells, we have generated mice lacking apolipoprotein C-III (ApoC-III). Homozygous mutant animals show absence of ApoC-III protein and no expression of ApoC-III mRNA in the liver or in the intestine. Expression of the neighboring genes, coding for apolipoprotein A-I and apolipoprotein A-IV, are not altered in the liver but are reduced in the intestine. This suggests that these three genes share a tissue-specific element for intestinal expression and that insertion of an additional promoter for the neomycin-resistant gene into the locus affects interaction between the tissue-specific element and the promoter of the individual gene. Fasted plasma triglyceride levels in the homozygous mutants are reduced to about 70% of normal, while heterozygotes have values intermediate between those of the homozygous mutants and wild types. Plasma levels of total cholesterol and of high density lipoprotein cholesterol in homozygotes are consistently lower than those in normal mices but the reduction does not reach statistical significance. A fat meal test showed that postprandial hypertriglyceridemia is abolished in homozygotes lacking ApoC-III. The homozygous mutants also clear chylomicrons faster than wild type controls. These data indicate that ApoC-III modulates the catabolism of triglyceride-rich lipoproteins and plays a role in the postprandial management of triglycerides.

PMID: 8089130 [PubMed - indexed for MEDLINE]

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1: J Nutr. 1991 May;121(5):653-62.

Related Articles, Links

Changes in rat plasma apolipoproteins and lipoproteins during moderate protein deficiency: potential use in the assessment of nutritional status.

Gouache P, Le Moullac B, Bleiberg-Daniel F, Aubert R, Flament C.

Unite de Recherches sur la Nutrition et l'Alimentation, U.1 INSERM, Hopital Bichat, Paris, France.

To test apolipoprotein sensitivity as protein deficiency markers, concomitant evolution of plasma apolipoproteins (apo) and usual nutritional markers (transthyretin, albumin, transferrin) were followed during a 28-d protein restriction in young male Wistar rats. In addition, plasma lipids and chemical composition of lipoproteins were assayed by d 28. The control and the deficient groups were fed 18% and 6% casein diets, respectively. By d 28 the protein-deficient group exhibited hypotriglyceridemia resulting from the decrease in VLDL triacylglycerols; free cholesterol and phospholipids were increased, reflecting the increment in LDL-HLDL1. In plasma total lipoproteins, apo BH, AI and E were not different than controls in the deficient group. Apolipoprotein AIV decreased after d 14 and was significantly less than in controls at d 28. Apolipoprotein BI was considerably reduced by d 14 (43% less) and d 28 (52% less) compared with the control group. Apolipoproteins C + AII were significantly lower in the protein-deficient group by d 14 (43%). By d 28, VLDL apo C were decreased 60% by protein restriction. Transthyretin level was 20% lower in the protein-deficient group by d 7 but returned to control values by d 14. A moderately lower value was observed for albumin by d 7 and d 14 and for transferrin by d 28. These results indicate that, in this model, apo BI and C are more sensitive to protein depletion than usual nutritional markers.

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1: Biochim Biophys Acta. 1988 Jul 1;961(1):53-64.

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Serum lipoprotein and apolipoprotein profiles of the genetically obese ob/ob mouse.

Camus MC, Aubert R, Bourgeois F, Herzog J, Alexiu A, Lemonnier D.

INSERM U1, Hopital Bichat, Paris, France.

The lipid transport system of 3-month-old male C57BL/6J obese (ob/ob) mice was investigated. Serum lipoproteins were separated by density gradient ultracentrifugation and characterized by their chemical and electrophoretic properties as well as their relative apolipoprotein contents, defined according to molecular weight and charge. Obese, ob/ob mice exhibited a marked hyperlipoproteinemia resulting from large increases in low-density lipoproteins (LDL, d 1.021-1.058 g/ml) and high-density lipoproteins (HDL, d 1.058-1.137 g/ml), particularly, the HDL2 subclass (d 1.058-1.109 g/ml). This increase in lipoproteins was entirely responsible for their hypercholesterolemia and hyperphospholipidemia. By contrast, these obese mice had a net decrease in very-low-density lipoproteins (VLDL, d less than 1.016 g/ml) and intermediate-density lipoproteins (IDL, d 1.016-1.021 g/ml), which accounted for their moderate hypotriglyceridemia. The chemical composition of heterogeneous light LDL (d 1.021-1.040 g/ml and dense LDL (d 1.040-1.058 g/ml) overlapped by HDL-like particles was highly modified. These modifications consisted of increases in the percentages of cholesteryl ester and phospholipid and decreases in that of triacylglycerol. There were also marked changes in the relative values of the apolipoproteins of VLDL, but principally, IDL and LDL. IDL and light LDL were poorer in apolipoproteins BH (Mr 340,000-320,000) and eventually in apolipoprotein BL (Mr 220,000-200,000) and enriched in apolipoproteins E (Mr 37,000-35,000) and C-A-II (Mr approximately equal to 12,000). A similar and very significant change occurred in VLDL for both the apolipoproteins BL and C-A-II. Dense LDL, mainly poorer in apolipoprotein BH and enriched in apolipoprotein A-I (Mr 28,000-27,000), closely resembled HDL2 in all the groups, and were enriched in apolipoproteins C-A-II in only the obese mice. We suggest that ob/ob mice are probably protected against atheromata because of the low VLDL and IDL levels, and the increase in HDL2.

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1: J Steroid Biochem Mol Biol. 2004 Jul;91(3):147-55.

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Estrogen-like activity of licorice root constituents: glabridin and glabrene, in vascular tissues in vitro and in vivo.

Somjen D, Knoll E, Vaya J, Stern N, Tamir S.

Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center and The Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 64239, Israel. dalias@tasmc.health.gov.il

Post-menopausal women have higher incidence of heart diseases compared to pre-menopausal women, suggesting a protective role for estrogen. The recently Women's Health Initiative (WHI) randomized controlled trial concluded that the overall heart risk exceeded benefits from use of combined estrogen and progestin as hormone replacement therapy for an average of five years among healthy postmenopausal US women. Therefore, there is an urgent need for new agents with tissue-selective activity with no deleterious effects. In the present study, we tested the effects on vascular tissues in vitro and in vivo of two natural compounds derived from licorice root: glabridin, the major isoflavan, and glabrene, an isoflavene, both demonstrated estrogen-like activities. Similar to estradiol-17beta (E2), glabridin (gla) stimulated DNA synthesis in human endothelial cells (ECV-304; E304) and had a bi-phasic effect on proliferation of human vascular smooth muscle cells (VSMC). Raloxifene inhibited gla as well as E2 activities. In animal studies, both intact females or after ovariectomy, gla similar to E2 stimulated the specific activity of creatine kinase (CK) in aorta (Ao) and in left ventricle of the heart (Lv). Glabrene (glb), on the other hand, had only the stimulatory effect on DNA synthesis in vascular cells, with no inhibition by raloxifene, suggesting a different mechanism of action. To further elucidate the mechanism of action of glb, cells were pre-incubated with glb and then exposed to either E2 or to gla; the DNA stimulation at low doses was unchanged but there was abolishment of the inhibition of VSMC cell proliferation at high doses as well as inhibition of CK stimulation by both E2 and by gla. We conclude that glb behaved differently than E2 or gla, but similarly to raloxifene, being a partial agonist/antagonist of E2. Glabridin, on the other hand, demonstrated only estrogenic activity. Therefore, we suggest the use of glb with or without E2 as a new agent for modulation of vascular injury and atherogenesis for the prevention of cardiovascular diseases in post-menopausal women. Copyright 2004 Elsevier Ltd.

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Effects of 3-phenyl-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]- 2H-1-benzopyran-7-ol (CHF 4056), a novel nonsteroidal estrogen agonist/antagonist, on reproductive and nonreproductive tissue.

Galbiati E, Caruso PL, Amari G, Armani E, Ghirardi S, Delcanale M, Civelli M.

Department of Pharmacology, Chiesi Pharmaceuticals S.p.A., Parma, Italy.

We have discovered a new, nonsteroidal, estrogen agonist/antagonist, 3-phenyl-4-[[4-[2-(1-piperidinyl)ethoxy] phenyl] methyl]-2H-1-benzopyran-7-ol (CHF 4056). The aim of this study was to determine the effects of CHF 4056 on a series of parameters (body weight, uteri, serum cholesterol, and bones) that were previously shown to be sensitive to estrogens and to selective estrogen receptor modulators (SERMs). CHF 4056 is a benzopyran derivative that binds with high affinity to the human estrogen receptors alpha and beta (dissociation constant K (i) of 0.041 and 0.157 nM, respectively). In immature rats, CHF 4056 induced a full estrogen antagonism (half-maximal efficacious dose = 0.33 mg/kg x day p.o.) coupled with a lack of uterine stimulatory activity, whereas the structurally related SERM levormeloxifene demonstrated a maximal partial agonist effect of approximately 65% that of 17alpha-ethynyl estradiol (EE2). In ovariectomized (OVX) rats, CHF 4056 (0.1-1 mg/kg x day p.o. for 4 weeks) significantly reduced OVX-induced bone loss in the lumbar spine L1-4 and OVX-induced increase in serum osteocalcin. These protective effects on bone tissue were comparable with those of 0.1 mg/kg x day EE2. In the same experimental conditions, serum cholesterol was significantly lower in the CHF 4056-treated animals, compared with vehicle-treated OVX rats. In line with the results observed in immature rats, also in OVX rats CHF 4056 diverged dramatically from EE2 and levormeloxifene in its lack of significant estrogenic effects on uterine tissue. In conclusion, CHF 4056 is a new SERM that produces beneficial effects on bone and cholesterol levels, while maintaining antagonist effects on the uterus.

PMID: 11861784 [PubMed - indexed for MEDLINE]

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1: J Mol Biol. 2002 Jan 11;315(2):229-38.

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Critical role of helix 12 of the vitamin D(3) receptor for the partial agonism of carboxylic ester antagonists.

Vaisanen S, Perakyla M, Karkkainen JI, Steinmeyer A, Carlberg C.

Department of Biochemistry, University of Kuopio, Finland.

The carboxy-terminal alpha-helix of a nuclear receptor ligand-binding domain (LBD), helix 12, contains a critical, ligand-modulated interface for the interaction with coactivator proteins. In this study, using the example of the vitamin D receptor (VDR) and the partial antagonist ZK159222, the role of helix 12 (residues 417-427) for both antagonistic and agonistic receptor actions was investigated. Amino acid residue G423 was demonstrated to be critical for partial agonism of ZK159222, but not for the activity of the natural VDR agonist, 1alpha,25-dihydroxyvitamin D(3) (1alpha,25(OH)(2)D(3)). The amount of partial agonism of ZK159222 increased when helix 12 was truncated by the last four amino acid residues (Delta424-27) and augmented even more, when in addition helix 12 of VDR's dimerization partner, retinoid X receptor (RXR), was truncated. In contrast, the low agonism of a structural derivative of ZK159222, ZK168281, was not affected comparably, whereas other close structural relatives of ZK159222 even demonstrated the same agonistic activity as that of 1alpha,25(OH)(2)D(3). The amount of agonism of ZK159222 and ZK168281 at different variations of helix 12 correlated well with VDR's ability to complex with coactivator proteins and inversely correlated with the strength of the compound's antagonistic action on 1alpha,25(OH)(2)D(3) signalling. Molecular dynamics simulations of the LBD complexed with the two antagonists could explain their different action by demonstrating a more drastic displacement of helix 12 through ZK168281 than through ZK159222. Moreover, the modelling could indicate a kink of helix 12 at amino acid residue G423, which provides the last four amino acid residues of helix 12 with a modulatory role for the partial agonism of some VDR antagonists, such as ZK159222. In conclusion, partial agonism of a VDR antagonist is lower the more it disturbs helix 12 in taking the optimal position for coactivator interaction. Copyright 2002 Academic Press.

PMID: 11779241 [PubMed - indexed for MEDLINE]

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1: Mol Endocrinol. 2000 Nov;14(11):1788-96.

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25-Dehydro-1alpha-hydroxyvitamin D3-26,23S-lactone antagonizes the nuclear vitamin D receptor by mediating a unique noncovalent conformational change.

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(23S)-25-dehydro-1alpha-Dihydroxyvitamin D3-26,23-lactone (TEI-9647; MK) has been reported to antagonize the 1alpha,25-dihydroxyvitamin D3 nuclear receptor (VDR)- mediated increase in transcriptional activity. Using a transient transfection system incorporating the osteocalcin VDRE (vitamin D response element) in Cos-1 cells, we found that 20 nM MK antagonizes VDR-mediated transcription by 50% when driven by 1 nM 1alpha,25(OH)2D3. Four analogs of 1alpha,25(OH)2D3, also at 1 nM, were antagonized 25 to 39% by 20 nM MK. However, analogs with 16-ene/23-yne or 20-epi modifications, which have a significantly lower agonist ED50 for the VDR than 1alpha,25(OH)2D3, were antagonized by 20 nM MK only at 100 pM or 10 pM, respectively. One possible mechanism for antagonism is that the 25-dehydro alkene of MK might covalently bind the ligand-binding site of the VDR rendering it inactive. Utilization of a ligand exchange assay, however, demonstrated that MK bound to VDR is freely exchanged with 1alpha,25(OH)2D3 in vitro. These data support the apparent correlation between VDR transcriptional activation by agonists and the effective range of MK antagonism by competition. Furthermore, protease sensitivity analysis of MK bound to VDR indicates the presence of a unique conformational change in the VDR ligand-binding domain, showing a novel doublet of VDR fragments centered at 34 kDa, whereas 1alpha,25(OH)2D3 as a ligand produces only a single 34-kDa fragment. In comparison, the natural metabolite 1alpha,25-dihydroxyvitamin D3-26,23-lactone yields only the 30-kDa fragment that is produced by all ligands to varying degrees. Collectively, these results support that MK is a potent partial antagonist of the VDR for 1alpha,25(OH)2D3 and its analogs when in appropriate excess of the agonist.

PMID: 11075812 [PubMed - indexed for MEDLINE]

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1: Proc Soc Exp Biol Med. 1998 Jan;217(1):45-52. Related Articles, Links

Selective estrogen receptor modulators: an alternative to hormone replacement therapy.

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Estrogen is a key regulatory hormone, which in addition to its role in reproduction, affects a number of physiological systems, including the skeleton and cardiovascular system. The important role of estrogen in various tissues is perhaps most evident in postmenopausal women who, in addition to menopausal symptoms, experience increases in osteoporosis and coronary heart disease as their estrogen levels decline. Estrogen replacement, while effective against osteoporosis and heart disease, produces a number of side effects associated with the breast and uterus which limits compliance. Selective estrogen receptor modulators (SERMs), such as raloxifene and tamoxifen, produce beneficial estrogen-like effects on bone and lipid metabolism, while antagonizing estrogen in reproductive tissue. SERMs can be distinguished from each other in reproductive tissue, particularly the uterus, by their activity profile. For example, while triphenylethylenes like tamoxifen behave as partial agonists, raloxifene (a benzothiophene) behaves as a complete antagonist in the uterus. The SERM profile is distinct from that of full estrogens (ie. 17beta-estradiol or 17alpha-dihydroequilenin) which behave as estrogen agonists in all tissues and pure estrogen antagonists (i.e. ICI-164,384) which exhibit only an estrogen antagonist profile in a battery of tissue types. The precise mechanism by which SERMs produce this tissue-selective pharmacology remains a question. It is clear, however, that for raloxifene, both the estrogen agonist effects on bone and cholesterol metabolism as well as the estrogen antagonist effects in uterine and mammary tissue involve high affinity interaction with the estrogen receptor. The estrogen antagonist activity is mediated via classical pharmacological competition for estrogen receptor binding. The estrogen agonist activity, in bone for example, appears to involve novel post-receptor pathways and non-classical estrogen response element(s) which are activated by SERMs. These novel response elements may represent natural pathways which respond to estrogen metabolites in vivo.

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PMID: 9421206 [PubMed - indexed for MEDLINE]

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1: J Biol Chem. 2004 Nov 16; [Epub ahead of print]

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Adipocytic differentiation and liver X receptor pathways regulate the accumulation of triacylglycerols in human vascular smooth muscle cells.

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Lipid accumulation by vascular smooth muscle cells (VSMC) is a feature of atherosclerotic plaques. In this study we describe two mechanisms whereby human VSMC foam cell formation is driven by de novo synthesis of fatty acids leading to triacylglycerol (TG) accumulation in intracellular vacuoles, a process distinct from serum lipoprotein uptake. VSMC cultured in adipogenic differentiation medium accumulated lipid and were induced to express the adipocyte marker genes adipsin, adipocyte fatty acid binding protein, C/EBPalpha, PPARgamma and leptin. However, complete adipocyte differentiation was not observed as numerous genes present in mature adipocytes were not detected and the phenotype was reversible. The rate of lipid accumulation was not affected by PPARgamma agonists but screening for the effects of other nuclear receptor agonists showed that activation of the Liver X Receptors (LXR) dramatically promoted lipid accumulation in VSMC. Both LXRalpha and LXRbeta were present in VSMC, and their activation with TO901317 resulted in induction of the lipogenic genes fatty acid synthetase, SREBP1c and stearoyl-CoA desaturase. 27-hydroxycholesterol, an abundant oxysterol synthesised by VSMC acted as an LXR antagonist, and therefore may have a protective role in preventing foam cell formation. Immunohistochemistry showed that VSMC within atherosclerotic plaques express adipogenic and lipogenic markers suggesting these pathways are present in vivo. Moreover, the development of an adipogenic phenotype in VSMC is consistent with their known phenotypic plasticity and may contribute to their dysfunction in atherosclerotic plaques and thus impinge on plaque growth and stability.

PMID: 15548517 [PubMed - as supplied by publisher]

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